REMARKS

Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 06-1050.

An unexecuted copy of DECLARATION 5 under 37 C.F.R. § 1.132 of Stephen Fabijanski and an unexecuted copy of DECLARATION 6 under 37 C.F.R. § 1.132 of Gyula Hadlaczky were submitted with the Amendment mailed April 30, 2007 in response to the Office Action mailed March 30, 2006. The executed DECLARATION 5 of Fabijanski and the executed DECLARATION 6 of Hadlaczky are attached hereto. The executed original DECLARATION 5 is identical to the unexecuted copy. The executed original DECLARATION 6 is identical to the unexecuted copy.

DECLARATION 5

As discussed in the response, mailed April 30, 2007, the DECLARATION 5 of Fabijanski is provided to evidence the generation of plant SATACs using methods as taught in the instant application. The Declaration 5 details construction of plant SATACs in two distinct plant species, *Nicotiana* and *Brassica*. Declaration 5 shows that by following the teachings of the application as of its earliest filing date, plant SATACs can be generated and maintained in plant cells. It also it noted that Declaration 5 does not introduce the 334 bp tobacco sequence that was introduced as heterologous DNA in Declaration 4 (and Declarations 2 and 3). In neither instance, is such sequence used to generate a plant SATAC. The Arabidopsis rDNA sequence and the selectable marker used were the same in the studies in both Declarations. Hence, the Declaration 5 shows that the 334 bp sequence is not required to generate a plant SATAC.

Dr. Fabijanski is not an inventor of this application, he is a Ph.D. Since those of skill in the art typically have advanced degrees, Dr. Fabijanski, who has a Ph.D. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol. It is noted that he is an employee of Agrisoma, a company in which Chromos has an ownership interest, and which is a licensee of the instant application. In performing or directing the experiments in Declaration 5, he, and others under his direction and control, followed the teachings in the application.

Specifically, the Declaration 5 of Dr. Fabijanski demonstrates that by following the teachings in the application, plant SATACs can be generated by i) introducing a DNA fragment with a selectable marker into a plant cell (in this case either *Nicotiana* protoplasts or *Brassica napus* protoplasts); ii) growing the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant SATAC is produced; and iii) selecting a cell that contains a plant SATAC. The plant SATAC contains a plant centromere, as well as amplified pericentric DNA and the introduced heterologous DNA.

Heterologous DNA containing homology to the pericentric region of plant chromosomes, a selectable marker, either phosphinothricin N-acetyltransferase (PAT) gene or phosphinothricin acetyl transferase gene *(bar)*, were introduced into *Nicotiana* protoplasts or *Brassica napus* protoplasts, respectively. Following selection of cells, fluorescence in situ hybridization (FISH) was used to demonstrate the formation of a sausage chromosome, and a resulting plant SATAC in both species of plants. As set forth in the Declaration 5, no knowledge of the plant centromere sequence, nor any plant sequence, is required. As taught in the above-captioned application, the plant SATACs were generated following amplification and the generation of a *de novo* centromere that occurs upon integration of a DNA fragment into the pericentric heterochromatin. The results of these analyses demonstrate that the methods described in the above-referenced application can be used to generate, identify and maintain plant SATACs in plant cells as taught in the application, including the ultimate parent application.

DECLARATION 6

As discussed in the response, mailed April 30, 2007, the DECLARATION 6 of Hadlaczky is provided to evidence the universality of the underlying chromosomal processes involved in the generation of SATACs and of the process for production of SATACs described in the application and the SATACs described in the application. The Declaration demonstrates that by following the teachings of the application, SATACs from diverse species, plants and mammals, have been prepared by the methods taught in the specification. The Declaration 6 demonstrates that the methodology generically described in the application and exemplified with rodent chromosomes, is reproducible. Further, the Declaration 6 points out that the methods disclosed in the application are based an universal amplification events common to all eukaryotic chromosomes. Based on data provided in the Declaration 6 and the

accompanying Declaration 5 of Fabijanski, the universality is evidenced by the fact that SATACs can be prepared in accord with the teachings of the application in species as diverse as mammalian species and plant species. Surely, if the amplification occurs in plants, humans and rodents, it is can be inferred that it is a universal phenomena that occurs in other mammals, including whales and gorillas and dolphins and rats and apes.

The Declaration 6 describes the results of the use of methods taught in the above-captioned application for the generation of satellite artificial chromosomes from human chromosomes. These results demonstrate that the methods of artificial chromosome production taught in the above-captioned application are broadly applicable and can be used to generate satellite artificial chromosomes from varied species of organisms.

Specifically, the Declaration 6 of Hadlaczky describes the generation of satellite artificial chromosomes from human chromosomes through the introduction of foreign DNA including a selectable marker into human/hamster hybrid cells containing human chromosomes. This Declaration 6 also incorporates Declaration 5 of Fabijanski, showing the generation of SATACs in plants.

In the demonstration described in Declaration 6, the hybrid recipient cell line, referred to as 94-3 cells, is a fusion of human lymphoblasts and Chinese hamster ovary cells and contains human and hamster chromosomes. Thus, as described in the above-captioned application, cells for use in generating artificial chromosomes can be any variety of cells and are not limited to mouse cells.

As also described in the Declaration 6, the 94-3 cells were transfected using standard calcium phosphate DNA precipitation methods with foreign DNA that included a selectable marker gene. The selectable marker gene was the puromycin N-acetyl transferase gene, which is among the several exemplary selectable markers referred to in the subject application. The remainder of the foreign DNA that was introduced into the 94-3 cells included the β-galactosidase gene, the expression of which may be easily detected, and mouse rDNA. The transfectants were cultured under selective conditions (*i.e.*, in the presence of puromycin), and cells that contain artificial chromosomes were selected in accordance with the methods set forth in the subject application. Specifically, using standard analytical techniques (*i.e.*, Southern hybridization, LacZ staining, C-banding and *in situ* hybridization) as taught in the application, the selected cells were analyzed to identify those that contain artificial chromosomes.

Declaration 6 describes the results of such analyses that were used to identify, for example, a human satellite artificial chromosome and a human gigachromosome, which is a precursor thereof, in the selected transfectants. Southern hybridization results revealed that greater than 40% of the transfectants contained a high copy number of the pBabe Puro (described in the instant application in Example 12) construct DNA which indicates an amplification of the integrated construct such as can occur in the development of an artificial heterochromatic chromosome. Greater than 30% of the transfectants also expressed β-galactosidase at levels detectable by LacZ staining.

To detect the amplified heterochromatic regions of any of the transfectant chromosomes that had undergone a large-scale amplification following introduction of the foreign DNA, C-band staining of the cells was conducted to specifically visualize constitutive heterochromatin. The results of C-banding analyses revealed that 30% of the transfectants contained amplified heterochromatic segments. In this manner, it was possible to detect sausage chromosomes with a characteristic extended heterochromatic arm just as described in the above-captioned application (see, *e.g.*, page 72, line 28, through page 73, line 4, of the application). *In situ* hybridization of selected transfectant cells with human genomic DNA and human alpha satellite DNA probes confirmed that the sausage chromosome was formed on a human chromosome. Further *in situ* hybridization studies of these cells using pBabe Puro and rDNA probes indicated that these foreign genes co-amplified in the heterochromatic arm of the sausage chromosome as is described in the above-captioned application in general and in the specific example of the analysis of a mouse sausage chromosome (see, *e.g.*, Example 4 at page 83).

Similarly, Declaration 6 describes the identification of human satellite artificial chromosomes and precursors thereof in the transfected cells. C-band staining of the selected transfectants revealed satellite artificial chromosomes containing two heterochromatic arms resulting from continued amplification of a sausage chromosome. *In situ* hybridization of the selected transfectants with human alpha satellite DNA and pBabe Puro probes confirmed that the artificial chromosome had human origins and contained the integrated foreign DNA. Thus, the results of these analyses are as described in the subject application in general and in the specific example of the analysis of a mouse megachromosome. *In situ* hybridization of the selected transfectants with a pBabe Puro probe also revealed the presence of a human

gigachromosome showing characteristics such as those described in the above-captioned application in general and in the specific example of the mouse gigachromosome.

Therefore, the Declaration 6 demonstrates that the disclosure in the above-captioned application provides methods for production of satellite artificial chromosomes in any species and provides satellite artificial chromosomes from varied species, thereby, demonstrating that the unsupported assertion in the Office Action that the specification is enabling only for mammalian, particularly mouse, satellite artificial chromosomes, is not correct. Further, it demonstrates that the process is universal and reproducible and can be successfully applied to many species, including plants. As stated in the Declaration 6 "the process by which SATACs are generated is a universal process, fundamental to replication and recombination in cells." In fact, Dr. Hadlaczky was awarded the prestigious Széchenyi award in 2000 for the work that is the subject of the above-captioned application.

Declaration 6 demonstrates element-for-element and step-for-step that, by following the teaching in the application, one of skill in the art can, without undue experimentation, introduce any heterologous DNA that into any cell, and generate and identify any resulting cells that can contains a satellite artificial chromosome.

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In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,

Stephanie L. Seidman
Reg. No. 33,779

Attorney Docket No. 17084-004006 / 402E
Address all correspondence to:
Fish & Richardson P.C.
12390 El Camino Real
San Diego, California 92130
Telephone: (858) 678-4777
Facsimile: (202) 626-7796
Email: seidman@fr.com